Identification of a new gene encoding pericentromeric dodeca-satellite binding protein in *Drosophila melanogaster*

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Abstract Dodeca-satellite (CCCGTACTCGGT)n is a type of tandemly repeated DNA sequence located in the pericentromeric region of the third chromosome of *Drosophila melanogaster* and that cross-hybridizes with DNA from other species such as *Arabidopsis*, mouse and human. This evolutionary conservation suggests that dodeca-satellite might play an important role in the centromeric function. Therefore, the aim of our research was the isolament of genes encoding proteins that might help stabilize these DNA structures, in vivo. To identify *D. melanogaster* sequence DNAs encoding dodeca-satellite binding proteins, we used the in vivo yeast assay, known as 'one-hybrid system'. Here, we identified a novel gene sequence that encoded pericentromeric dodeca-satellite binding protein and described its sequence characteristics.

Key words: Dodeca-satellite; DNA binding protein; *Drosophila melanogaster*

1. Introduction

The genome of higher eukaryotes contains large amounts of simple and complex tandemly repeated DNA sequences, termed satellite DNA.

During interphase, eukaryotic chromatin is divided into regions that differ in degrees of compaction: the transcriptionally active euchromatin and the more compact heterochromatin [1]. The ubiquity of heterochromatin has prompted much speculation about its function including a genetic dumping ground maintained by unequal exchange, a requirement for structural isolation of euchromatin from centromeres, and an initiation site for chromosome condensation [2].

In *Drosophila melanogaster* (*D.m.*), heterochromatin accounts for about 29% and 35% of the aploid genome in females and males, respectively [3]. In this organism, as in many others, heterochromatic regions are known to contain the centromeres, the sequence involved in kinetochore formation and pairing between sister chromatids. Chromatin and kinetochore structures undergo profound changes during cell cycle progression. The primary construction contains the spindle fiber attachment region, a key component of the mechanisms for chromosome segregation whose structure and molecular nature remain largely unknown.

Models of the centromeres of higher eukaryotes predict that centromere-associated satellite DNA is necessary for centromere functionality [4–6].

Many observations emphasize the role of centromeric satellite DNA in the structure and function of the centromere, such as stable curvatures [7,8], that are involved in protein binding capability [9] and the presence in satellite DNAs of conserved sequence motives.

The search for a heterochromatin role in *D.m.* meiosis has been fruitful. In most eukaryotes, proper meiotic segregation is ensured by euchromatic pairing, chiasma formation and recombination. Meiotic pairing has been shown to require heterochromatic homology and to be sensitive to repeat number in both male and female *D.m.* In two genetically distinct achiasmate systems in *D.m.*, repeated sequences in the pericentric heterochromatin have been shown to pair homologues for faithful meiotic segregation. The accumulation of tandem repeat elements increases pairing ability of repeated sequences. This is one of the reasons to define a general role for heterochromatin [10].

In *D.m.*, only one centromere binding protein, zw10, has been identified. This transiently binds to centromeres during anaphase, and is required for proper chromatidic segregation [11].

In human and mouse, centromere binding protein, CENP-B plays an important role in the assembly of specific centromere structures by forming unique DNA-protein complexes at the CENP-B box sites on the centromeric repetitive DNA in interphase nuclei and in mitotic chromosomes [12].

A biochemical procedure has been designed for *D.m.* to search for functional homologues of CENP-B and the data suggest that at least one of the three isolated proteins might be a functional homologous of the human CENP-B. However, immunolabeling of early embryos using a polyclonal serum raised against all three proteins indicates that they localize preferentially to chromosomal regions containing constitutive heterochromatin. During metaphase and anaphase the antigens identified by the serum accumulate near and at the centromere, but the label does not appear to localize exclusively to heterochromatin since throughout different mitotic stages a faint overall label of chromosome arms is present [13].

The comparison of DNA sequence motifs, organization and kinetochore components from yeast to man is beginning to indicate that, although centromeres are highly variable elements, a conserved sequence pattern, function and organization are emerging. Shared motifs amongst kinetochore components in yeast and man suggest that mechanisms of segregating sister chromatids are conserved.

Alpha-satellite DNA, like many other centromeric satellite DNAs, evolved very rapidly and sequence comparison between different organisms shows little similarity. On the contrary, it would be expected that features of centromere structure that are important for function may be conserved through evolution. Therefore, it seems reasonable that other classes of DNA sequences and proteins are involved in the

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formation of a fully functional centromere [14]. In this respect, it is important to notice that the centromeric human 5 bp satellite 3 and the *D.m.* pericentric dodeca-satellite appear to be conserved [15]. The most important feature of dodeca-satellite sequences is its close proximity to the primary construction and it can form connections between sister chromatids during mitosis [16]. For this reason, these sequence could offer a valuable tool toward the characterization of this region. Therefore, the discovery of dodeca-satellite binding proteins is biologically important, because they might be evolutionary conserved and could help us to understand the requirements for the centromeric function.

We here identified cDNA clones encoding dodeca-satellite binding activity using a genetic selection in yeast, and isolated a novel gene we called GV1.

2. Materials and methods

2.1. Yeast one-hybrid screening of the D. melanogaster embryo cDNA library

Six copies of the dodeca-satellite pericentromeric sequence were used as the bait to select DNA binding domains encoded in the *D.m.* embryo cDNA library. The bait was inserted into reporter plasmids pHISi and pLacZi, and the recombinant plasmids were introduced sequentially into the genome of the yeast strain Y4271 to create yeast reporter strains. These plasmids were supplied by Clontech Matchmaker One-Hybrid System kit.

Transformation was carried out by using 20 μg of a cDNA library fused with yeast GAL4 activation domain derived from *D.m.* embryos (Clontech) by LiAc-polyethylene glycol. Yeast transformants were plated onto 20 SD/−Leu/−His/+50 mM 3-amino 1,2,4-triazole plates and incubated at 30°C for 6 days. A total of 20 transformants grown on His- and Leu-selective medium were recovered and they were tested for β-galactosidase activity. Plasmids from putative positive clones were isolated from the yeast after homogenization with glass beads and then individually transferred into DH5α cells for amplification. To eliminate false positive, these plasmids were separately introduced into yeast cells containing six copies of the dodeca-satellite pericentromeric sequence and the transformants were tested for β-galactosidase activity. Only the plasmids that conferred positive expression were analyzed. Clones containing cDNA for possible DNA binding proteins of interest were screened by PCR to test the size of the inserts. The amplification products were analyzed in 1% agarose gel, fragments were eluted from the gel and directly sequenced.

cDNA sequence analysis of both strands was determined by the Big Dye Terminator Cycle Sequencing method (ABI-PRISM Sequencer 310 Perkin-Elmer).

DNA binding protein homology was analyzed by the BLAST Network Service at NCBI. The secondary structure of Gv1 protein was predicted by Robson-Garnier secondary structure algorithms.

2.2. Gv1 domain mapping

The Gv1 binding domain was identified by using the yeast one-hybrid system and confirmed by EMSA. To test for binding domain in Gv1 protein, constructs with internal deletions of sequences from restriction sites encoding proteins with deletions in separate regions (Gv1Δ 14–61; Gv1Δ 62–125 and Gv1Δ 126–180) were cloned in pGAD424 plasmid (Clontech) and transformed into dodeca-satellite bait, containing yeast cells. The transformants were tested for growth ability on His and Leu plates with 50 mM 3-AT.

2.3. Band-shift by EMSA

To confirm DNA binding activity, we performed gel-shift DNA binding assays. The GV1 cDNA was transferred to pGEM Easy plasmid (Promega). The protein was synthesized by using 1 μg of linearized plasmid and the coupled reticulocyte lysate kit (Roche-Boehringer). EMSA was carried out by using in vitro translated protein.

One μl of protein was added to 20 μl binding reaction (binding buffer 5×the following: 5 mM DTT, 1% Tween 20, 100 mM HEPES, 5 mM EDTA, 50 mM $(NH)_4SO_4$, 150 mM KCl; and 1 μg Poly dI-dC was added to reaction mix). After a 10 min ice bath, 3′ end labeled (11-ddUTP Digoxigenin; Dig-Shift kit, Roche-Boehringer) double strand probe composed of six dodeca-satellite repeats was added to the binding reaction for 20 min incubation. To test for specificity of binding, competitor DNAs were added in a 200-fold molar excess. For specific competitor, we used an unlabeled oligonucleotide with the same sequence as dodeca repeats; as aspecific competitor $(AAAGTACTCAAT)_6$ was the unlabeled oligonucleotide. For protein controls, Oct2A and luciferase protein encoded from pGEM Easy plasmid (in vitro transcription and translation kit, Roche-Boehringer) were assayed in the EMSA.

To test for binding domain in Gv1 protein, proteins with deletions in separate regions (Gv1Δ 14–61, Gv1Δ 62–125 and Gv1Δ 126–180) were used in band-shift assays.

The mixtures were electrophoresed on a 5% polyacrylamide gel (59:1) in 0.5×TBE at 4°C. DNA was transferred onto nylon membrane by Southern blotting and crosslinked by baking the membrane for 30 min at 120°C. Immunological detection after hybridization was performed using an antibody conjugate (anti-digoxigenin-Dig-AP/Dig-Shift kit; Roche-Boehringer). A subsequent enzyme catalyzed color reaction with 5-bromo-4-chloro-3-indolyl phosphate (X-phosphate) and nitro blue tetrazolium salt (NBT) produced an insoluble blue precipitate, which visualized the hybrid molecules.

2.4. Southern analysis

Southern analysis using high molecular weight genomic DNA from *D. melanogaster* embryos was carried out using standard techniques. The filters were prehybridized, then hybridized ($T = 65$°C) with the random primer labeled with digoxigenin-dUTP 826 bp *D.m.* GV1 cDNA. Immunological detection after hybridization was performed as described previously.

3. Results

3.1. Cloning in vivo of specific dodeca-satellite binding proteins

The reported low abundance [18] of protein factors that recognize dodeca-satellite DNA structures, suggested that purification of such DNA binding proteins by biochemical methods is difficult.

Therefore, we used a cloning strategy using a genetic selection of *D.m.* embryo cDNAs encoding proteins that can activate a reporter construct in yeast.

The reporter construct consisted of six copies of the monomeric unit repeat of *D.m.* dodeca-satellite (CCCGTACTCGGT) adjacent to a low activity promoter directing HIS3 gene expression. The presence of this reporter is unable to recover the HIS3 phenotype of the parent yeast strains. We introduced into these cells a cDNA library of embryo *D.m.* genes fused to Gal-4 activation domain (AD). Yeasts that expressed a Gal-4 AD dodeca binding fusion protein capable of binding the six dodeca repeats present in the reporter, activated transcription of HIS3. We obtained 20 clones by screening that were positive for β-galactosidase activity. The plasmid from each transformant was extracted and individually reintroduced into yeast cells containing six dodeca repeats and the transformants were tested for β-galactosidase assay.

PCR analysis determined cDNA fragment size from library. Of these 20 clones, 14 contained inserts of about 1.1 kb, while the remaining inserts ranged from 750 to 1400 bp.

3.2. Nucleotide and predicted sequence of GV1

The longest open reading frame obtained from the 14 selected *D.m.* embryo cDNAs predicts a protein of 180 amino acids (Fig. 1).

To study the overall structure of the protein encoded by the GV1 cDNA, we determined its nucleotide and predicted pro-

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CGGCCGCGTCGACCTTCAATGAGTTTTGTCATGCTTTAGCGCAGAAACAGCGGTTCGATATCGAGA
TACCCACAGCATATAAACAGTATAATATACTATATCCTTCAGATACCAGATAGCAGCGGCAAACTAA
GCGACTTAGAAGTTTCGAGTTTCATTTGACCAAACACAATCGACACAAA
ATGCTCACTCCTTGCCTGCTGCTAGTCGCCACAGTCGCCAGTTTCAGCCTCCACGCGCAG

M  L  T  P  C  L  L  L  V  A  T  V  A  S  F  S  L  H  A  Q

GCCATCCGGGTGGACTGGGGCACCAACACAGGACCCATAGCACCACCTCCGCCGCGCACC

A  I  R  V  D  W  G  T  N  T  G  P  I  A  P  P  P  P  R  T

ACTCCGCAGCCGCCGAGGAAACCATACCCGGGATCCAACGCCCGTCTGGGAGGACCAGAC

T  P  Q  P  P  R  K  P  Y  P  G  S  N  A  R  L  G  G  P  D

GATGACGTACCCAATCCCAATCCCTATGTCTACGTGTTGCCACCGCCTTCGAGACCAAGG

D  D  V  P  N  P  N  P  Y  V  Y  V  L  P  P  P  S  R  P  R

ACCTGGGCGATTCCCGCCGGACCATATGCTCCGCCCAACTACAACAACCTGCCGCCCAAG

T  W  A  I  P  A  G  P  Y  A  P  P  N  Y  N  N  L  P  P  K

GGCAACAACTACGGCCAGCTGGCCAGCAATTCCTACAGCGGAGGAGTGACCTCCGTGCCG

G  N  N  Y  G  Q  L  A  S  N  S  Y  S  G  G  V  T  S  V  P

GGACTGGCCGCCAGTATGTGCCCGGAGTGGGCATCAAGTATACGGCAATTGTGTCTGATAAGC

G  L  A  A  Q  Y  V  P  G  V  G  I  K  Y  T  A  I  V  S  D  K

TGCAGGGCAAATACAATGCGAAGACCAAGAAGTATAAGGCCTATGAGAAGGCCAAGTACG

L  Q  G  K  Y  N  A  K  T  K  K  Y  K  A  Y  E  K  A  K  Y

CGTACCCCTGGAACTATGTAAGGCAATACGAGAATCGAAAAGCGATATCTGCGCTAT

A  Y  P  W  N  Y  V  R  Q  Y  E  N  R  K  R  Y  L  R  Y

TGAACCGCCGATACCCGATATGCATTTAGAACTACCTATATATAATTGTACAAGAGATTAAAGCCAG
ACACCTCAGACACGAAAAAAAAAAAAAAAAAAAAACCT
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Fig. 1. Sequence of GV1 cDNA and its predicted translation product. Stop codons at the end of the open reading frame are underlined. The regions that are thought to be kinase C phosphorylation and tyrosine kinase phosphorylation sites are boxed.

tein sequence. Using the BLAST Network Service at NCBI, this cDNA shows 98% homology with genomic sequence on chromosome 3L of *D.m.*, region 62A10-62B5, clone DS04808 from Berkeley Drosophila Genome Project, NID g3492862. This comparison allowed the mapping of the complete ORF found here, to the genomic DNA in this region. These results demonstrate that GV1 may contain a complete gene, although the 5′ end was not rigorously mapped. This cDNA was found to be 826 nucleotides in length, as shown in Fig. 1. The cDNA was sequenced completely on both strands and compared to the sequence of overlapping genomic DNA to determine the intron/exon structures and directions of transcription. The intron sequence for the gene was determined by comparing the genomic and cDNA sequence. The direction of transcription was determined by comparing the cDNA coding strand sequence with the orientation of the matching genomic gene sequence. The size of GV1 gene was confirmed by Southern analysis of restriction enzyme digested genomic DNA, using GV1 cDNA as probe (Fig. 2). Using *Hin*dIII, *Msp*I and *Hpa*II, we obtained the expected fragments (11 615 and 6599 bp for *Hin*dIII; 1060, 937, 575, 524 bp for *Msp*I and *Hpa*II) indicating that the gene is complete and is a single copy.

The methionine start codon is at position 183. GV1 cDNA appears to contain the entire ORF and it ends with a poly(A) tail. The ORF (Fig. 1) is 180 amino acids long and predicts a 19 851.57 Da molecular mass protein and a 10.14 isoelectric point. The BLAST Network Service at NCBI was searched to detect any similarity of Gv1 to other known proteins. No homology was found, therefore Gv1 is novel.

Secondary structure analysis of Gv1 protein predicted that amino acids 4–20 form an α-helix followed by a coil and a second α-helical region at amino acids 141–160. The presence of these regions suggests that Gv1 is able to counteract DNA sequences. Drosophila GV1 sequence possesses several motifs

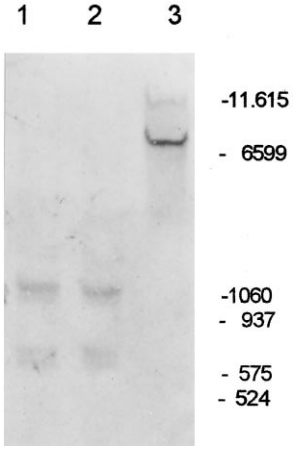

Fig. 2. Southern blotting analysis of GV1 gene. Genomic DNA was isolated from embryo flies, digested with *Msp*I and *Hpa*II (lanes 1 and 2), *Hin*dIII (lane 3), size fractionated in an agarose gel, transferred onto a filter, and probed with *D. melanogaster* GV1 cDNA.

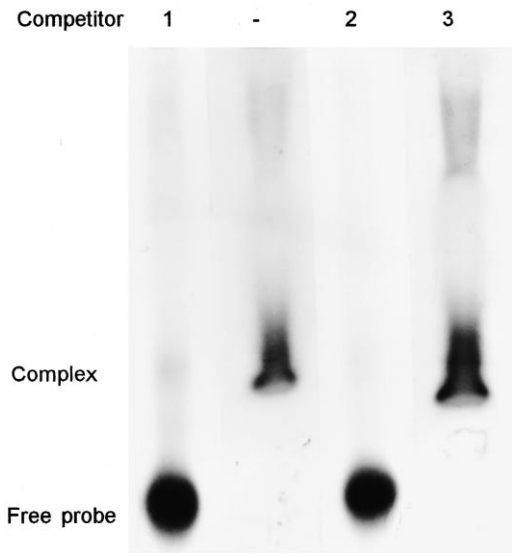

Fig. 3. EMSA was carried out with in vitro translated Gv1 protein. The probe represented six repeats of dodeca-satellite unit repeat, and, where indicated, a 200-fold molar excess of unlabeled competitor DNA fragments was included. Lanes: –, no competitor; 1, free probe; 2, dodeca, same as the probe sequence; 3, oligonucleotide A-T rich.

(PROSITE) such as two protein kinase C phosphorylation sites at residues 139–141 (SDK) and 150–152 (TKK), one tyrosine kinase phosphorylation site (KAYEKAKY) at residues 154–161 and four *n*-myristoylation sites at residues 101–106 (GNNYGQ), 105–110 (GQLASN), 114–119 (GGVTSV), 131–136 (GIKYTA).

3.3. DNA binding activity by EMSA

The dodeca-satellite binding properties of the protein extracted here, were assessed by electrophoretic mobility shift assay (EMSA). Double stranded oligonucleotides containing six copies of the monomeric unit repeat of *D.m.* dodeca-satellite were specifically shifted by the protein isolated here (Fig. 3). The formation of the protein-DNA complex is observed both as the appearance of the slower migrating DNA bands and as a decrease in the amount of the free probe. Using Oct2A and luciferase as control proteins we observed no formation of DNA-protein complex (Fig. 4); the migration pattern of the DNA-protein complex even in the presence of aspecific competitor did not change (Fig. 3), indicating a binding specificity. To map Gv1 binding domains we used one-hybrid system assays (Fig. 6), confirmed by band-shift assays using proteins with deletions of separate regions as shown in Fig. 5A and B. These constructs (Fig. 5A) constitute internal deletions of GV1 cDNA sequence. Deletion of sequence between amino acids 62–125 (Gv1Δ 62–125) did not reduce binding activity (Fig. 6, sector 3 and Fig. 5B, lane 3); deletion between amino acids 14–61 resulted in lower activity than that in full-length protein (Fig. 6, sector 4 and Fig. 5B, lane 4). Gv1Δ 126–180 was completely inactive when tested for growth under His^- selection and for band-shift assay (Fig. 6, sector 5 and Fig. 5B, lane 5). Therefore, sequence between amino acids 126–180 may be necessary to dodeca-satellite binding.

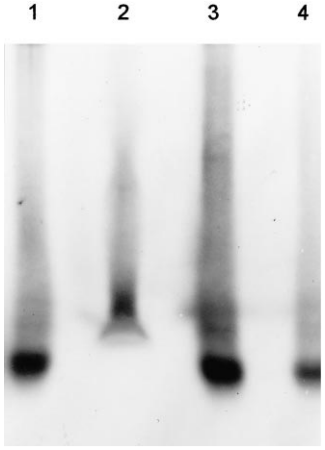

Fig. 4. EMSA was carried out with in vitro translated Gv1 and control proteins (Oct2A, luciferase). In all lanes the probe represented six copies of dodeca-satellite unit repeat. Lanes: 1, free probe; 2, Gv1; 3, luciferase; 4, Oct2A.

4. Discussion and conclusion

Our main source of knowledge about the molecular requirements of centromere structure and function has come from the study of functional centromeric sequences isolated from the yeast *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*.

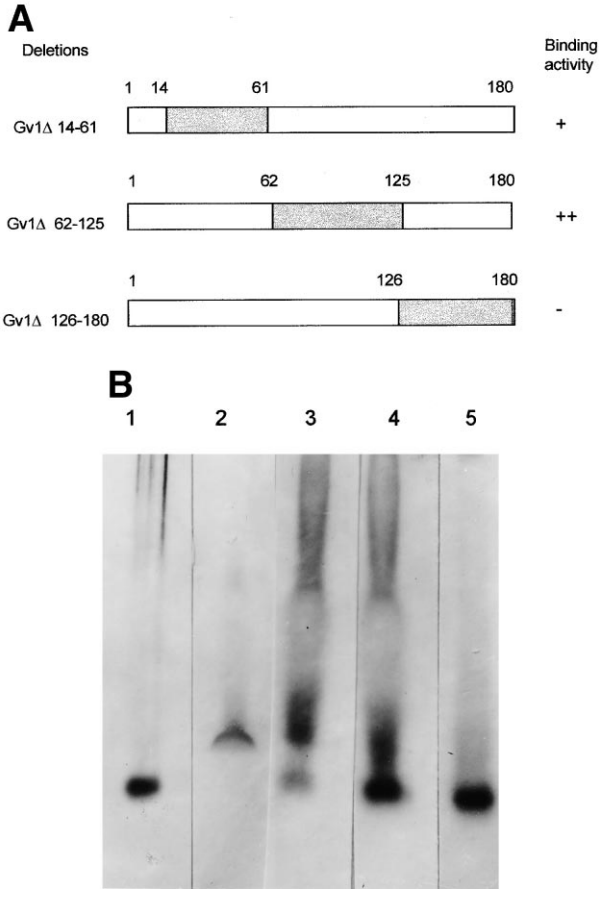

Fig. 5. A: Constructs encoding Gv1 mutated by internal deletions. B: EMSA was carried out with in vitro translated deleted Gv1. In all lanes the probe represented six copies of dodeca-satellite unit repeat. Lanes: 1, free probe; 2, Gv1; 3, Gv1Δ 62–125; 4, Gv1Δ 14–61; 5, Gv1Δ 126–180.

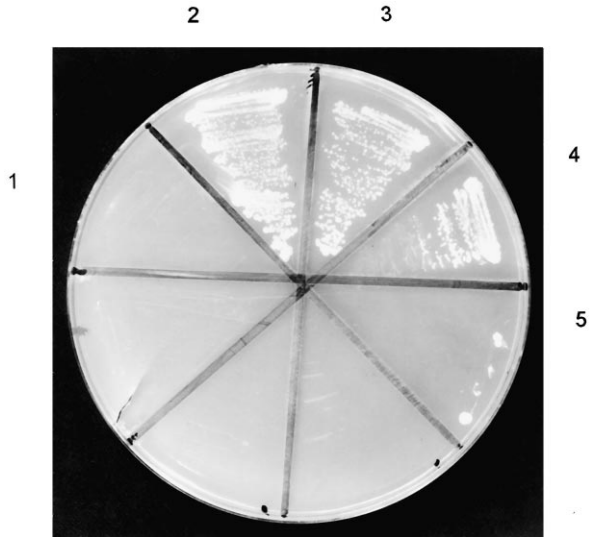

Fig. 6. Mapping of Gv1 protein by the yeast one-hybrid system. Growth of yeast cells under His selection transformed with pGAD424 expressing wild-type and deleted Gv1 proteins. 1, negative control transformed with plasmid with no insert cloned; 2, positive control with wild-type Gv1 cloned in pATC2; 3, Gv1Δ 62–125; 4, Gv1Δ 14–61; 5, Gv1Δ 126–180.

In higher eukaryotes, the centromeric DNA structure is very complex and the requirements for a functional centromere remain largely unknown [15]. The centromeric region is rich in satellite sequences that contribute to the centromere structure and the known centromeric satellite sequences are not evolutionary conserved, while the pericentromeric dodeca-satellite from *Drosophila* is conserved as between individuals amongst the same species although geographically distant strains *D.m.*, as amongst philogenetically distant organism [16]. Therefore, it is very likely that such sequences play a role in centromeric function, not only in *Drosophila*, but also in other organisms included humans. Therefore, finding dodeca-satellite binding proteins is biologically relevant, because they may be evolutionarily conserved and could help us to understand the requirements for centromeric function. *D.m.* dodeca-satellite sequences are closely linked to the centromere, within the pericentric heterochromatin of the right arm of the third chromosome. These sequences are detected as one or a few dots in the central region of the chromocenter in polytene chromosomes and during the cell cycle in untreated *D.m.* diploid cells. In interphase cells, hypotonic shock promotes the decondensation of the genomic region containing this satellite, resulting in a string-like structure. Following colchicine treatment, the dodeca-satellite sequence was shown within the space connecting sister chromatids [17].

Based upon these considerations, it is reasonable to think that the dodeca-satellite sequences play a role in the condensation and segregation of eukaryotic chromosomes preceding mitosis. Likewise, proteins that bind these sequences contribute to the stability and function of these sequences. It seems likely that protein factors anchored to these heterochromatic sequences cooperate to maintain the metaphase position of sister chromatids.

In this study we have demonstrated a binding activity between dodeca-satellite and a novel protein, confirming binding specificity by mobility-shift assays in vitro.

Until now it has not been possible to demonstrate a functional role for this DNA-protein complex, but it is interesting to note that Gv1 protein possesses two protein kinase C phosphorylation sites, perhaps it might be involved in the cell cycle. In fact, in eukaryotes numerous proteins are phosphorylated to promote the profound structural reorganization that accompanies the entry of cells into mitosis [18]. At the G2/M transition, the total amount of intracellular protein bound phosphates increases significantly, indicating that phosphorylation constitutes a major mechanism to bring about the profound structural reorganizations that accompany cell entry into mitosis. Typical mitotic events include chromosome condensation, disassembly of the nuclear lamina, breakdown of the nuclear envelope and reorganization of the microtubule network and formation of mitotic spindle. A molecular understanding of these processes remains a long-term goal, but it is very interesting that the spindle-associated kinesin-like motor protein CENP-E has been identified as a possible physiological substrate of CDC22/cyclin B [19]. One important question concerns the onset of anaphase: it has been proposed that sister chromatid separation may depend on the inactivation or proteolytic destruction of an as yet unidentified protein associated with chromosomes [20].

The finding of a dodeca-satellite binding protein may help us to realize the role of heterochromatic DNA-protein complex, to understand the meaning of repeated DNA in pericentromeric regions of chromosomes. It is important to notice that dodeca-satellite appears to be conserved amongst philogenetically distant organisms, therefore our finding could make a contribution to the knowledge about heterochromatic proteins in *D.m.* and provides the basis for identifying unifying principles in centromere structures across species.

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References

[1] John, B. (1988) in: R.S. Verma (Ed.), Heterochromatin: Molecular and Structural Aspect, Cambridge University Press, Cambridge, pp. 1–147.
[2] Pardue, M.L. and Hennig, W. (1990) Chromosoma 100, 3–7.
[3] Gatti, M., Pimpinelli, S. and Santini, G. (1976) Chromosoma 57, 351–375.
[4] Zinkowski, R.P., Meyne, J. and Brinkley alo, B.A. (1987) J. Cell. Biol. 50, 1101–1108.
[5] Vogt, P. (1990) Human. Genet. 84, 301–336.
[6] Vogt, P. (1992) Chromosoma 101, 585–589.
[7] Radic, M.Z., Lundgren, K. and Hamkalo, B.A. (1987) Cell 50, 1101–1108.
[8] Hamkalo, B.A., Lundgren, K., Radic, M.Z. and Saghbini, M. (1993) in: B.K. Vig (Ed.), Chromosome and Segregation and Aneuploidy, NATO ASI Series H72, Springer, Berlin, pp. 151–164.
[9] Hamkalo, B.A. (1992) Chromosoma 101, 602–608.
[10] Irick, H. (1994) Chromosoma 103, 1–3.
[11] Williams, B.C. (1994) J. Cell. Sci. 107, 785–798.
[12] Kitagawa, K., Masumoto, H., Ikeda, M. and Okazaki, T. (1995) Mol. Cell. Biol. 15, 1602–1612.
[13] Avides, M.C. and Sunkel, C.E. (1994) J. Cell Biol. 127, 1159–1171.
[14] Tyler-Smith, C. and Oakey, R.J. (1993) Nat. Genet. 5, 368–375.
[15] Abad, J.P. and Carmena, M. et al. (1992) Proc. Natl. Acad. Sci. USA 98, 4663–4667.
[16] Carmena, M., Abad, J.P., Villasante, A. and Gonzales, C. (1993) J. Cell Sci. 105, 41–50.
[17] Ferrer, N., Azorin, F., Villasante, A., Gutierrez, C. and Abad, J.P. (1995) 245, 8–21.
[18] Nigg, E.A. (1995) BioEssays 17, 471–480.
[19] Liao, L., Li, G. and Yen, T.J. (1994) Science 265, 394–398.
[20] Holloway, S.L., Glotzer, M., King, R.W. and Murray, A.W. (1993) Cell 73, 1393–1402.